IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of	Examiner: ANDERSON, James D.
BEDNARSKI *et al.*	Art Unit: 1614 Conf. No.: 7135
Application No. 10/681,855	CERTIFICATE OF ELECTRONIC TRANSMISSION UNDER 37 C.F.R. 1.8
Filed: October 7, 2003	I hereby certify that this correspondence, including listed enclosures, is being electronically transmitted to the United States Patent and Trademark Office in accordance with 37 C.F.R. 1.6(a)(4) on:
For: X-NITRO COMPOUNDS, PHARMACEUTICAL COMPOSITIONS THEREOF AND USES THEREOF	Dated: June 12, 2008
	Signed: Jennifer C. Black
	Jennifer C. Black

DECLARATION UNDER 37 CFR 1.132

I Ralph J. Bernacki hereby declare and state as follows:

1.   Attached as Exhibit 1 is an abbreviated form of my curriculum vitae. The following bullet points briefly summarize my experience in cancer research:

- PhD Pharmacology and Toxicology, University Rochester-1972
- Cancer Research Scientist-Roswell Park Cancer Institute (RPCI) 1972-
- Co-Director, New Drug Development Resource (RPCI)
- Interim Chair-PK/PD Drug Development Resource (RPCI)
- Member Scientific Research Committee-reviews all clinical protocols prior to review by the RPCI-Institutional Review Board
- Member of the Editorial Board of *Molecular Cancer Therapeutics*, an American Association of Cancer Research (AACR) sponsored journal
- Member of NIH/NCI Drug Development and Molecular Pharmacology (DMP) study section, charged with prioritization and funding of federal grant applications
- Author of over 150 peer reviewed journal articles and book chapters, focused in the area of cancer research and drug development

- University at Buffalo (UB), Professor and graduate course coordinator in Cancer Chemotherapy
- Major Professor and Mentor of over a dozen PhD and MS student thesis dissertations focused on various aspects of cancer drug development
- International lecturer and consultant in the area of cancer drug development, tumor metastasis and angiogenesis
- Patent holder for inventions related to cancer treatments.

I consider myself to be an expert in the discovery and development of new agents and treatments potentially useful for cancer therapy. My experience includes the evaluation of thousands of novel natural products and synthetic agents using *in vitro* high throughput screening technologies focused on the identification of lead agents with potential for the treatment of cancer in man. I have ample experience in the development and utilization of animal model systems useful for the preclinical evaluation of antitumor efficacy and toxicity of new agents. I am a translational scientist capable of bringing bench findings to the bedside, serving on various institutional, national and international committees focused on cancer drug development. My research studies have utilized human prostate, breast, lung, ovarian, hematological, colorectal, bladder, melanoma and liver xenograft tumor models, including drug resistant variants, leading to selection of novel agents for clinical trial.

2. I have reviewed: (1) the above identified patent application; (2) the Office Action mailed on September 20, 2007; (3) The Amendment After a Non-Final Rejection mailed March 20, 2008; and (4) selected portions of the provisional patent application attached to the Declaration of Richard F. Trecartin submitted with the March 20 Amendment (attached as Exhibit 6).

3. Attached hereto are Exhibits 2- 5 which were supplied to me by RadioRx Inc., the assignee of the above application.

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4.   Exhibit 2 sets forth the IC50 (mM) of cisplatin and various nitro containing compounds against HT29 cancer cells and their relative solubility. As can be seen, all of the compounds have a detrimental effect against HT29 cancer cells.

5.   Exhibit 3 sets for the same information for a subset of the compounds set forth in Exhibit 2. The subset represents those compounds with an IC50 less than 1 mM from Exhibit 2.

6.   There are nine dinitroazetidine compounds in Exhibit 3 which are identified in bold type. The activity of these compounds against HT29 cancer cells as a function of compound concentration as compared to cisplatin set is forth in Exhibit 4. The most potent compounds are ABDNAZ and IADNAZ. , Each of these compounds is more active than cisplatin against the HT29 cancer cells at a given concentration.

7.   Exhibit 5 sets forth the *in vitro* activity of ABDNAZ as compared to cisplatin against human glioblastoma cancer cells (SNB75 and U87); human prostate cancer cells (DU145); human colon cancer cells (HT29) and murine squamous cell carcinoma cells (SCC VII). I also note additional results in Exhibit 6 for a promyelocytic leukemic cell line (HL-60) as set forth in Examples 2, 3 and 4 and figures 4, 5 and 6. See Exhibit 6 – Pages 2, 3, 20, 21 and 22.

8.   In addition to the *in vitro* results identified above, I have also reviewed the experiment and results set forth at Exhibit 6 – Pages 9-11. These results clearly indicate that ABDNAZ is as effective as cisplatin against murine SCC VII tumor cells in a tumor-bearing mouse as indicated in the figure at Exhibit 6 – Page 10. However, the data in the figure in Exhibit 6 –Page 11 shows that ABDNAZ does not cause the loss of body weight, as demonstrated for cisplatin, which indicates that this treatment regime using ABDNAZ was well tolerated in rodents.

9.   The working examples in the present application (Serial No. 10/681,855) provide guidance on how to conduct *in vitro* and *in vivo* experiments, such as those set

forth above, against human tumor cell lines to determine the activity and efficacy of nitro containing compounds *See* e.g. pages 20-25 of the application.

10.   Based on the foregoing, it is my opinion that (1) the application provides sufficient disclosure to enable the skilled artisan to test and identify nitro compounds, such as ABDNAZ, that are effective against one or more cancer cell lines in *in vitro* and *in vivo* models and (2) there is a likelihood that such compounds can be used successfully in human clinical trials.

11.   I an aware that willful false statements and the like are punishable by fine or imprisonment or both (18 USC 1001), and may jeopardize the validity of the patent application or any patent issuing thereon.

Date: ___June 5, 2008_____   ___Ralph J. Bernacki_____
                                      Dr. Ralph J. Bernacki

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Principal Investigator/Program Director (Last, First, Middle):

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Ralph J. Bernacki	POSITION TITLE Member and Professor
eRA COMMONS USER NAME BERNACKI	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Rensselaer Polytechnic Institute, Troy, NY	B.S.	1968	Biology
U. of Rochester, School of Med., Rochester, NY	Ph.D.	1973	Pharmacology

Positions and Honors. List in chronological order previous positions, concluding with your present position. List any honors. Include present membership on any Federal Government public advisory committee.

Positions

1972-1974	Cancer Research Scientist I	Pharmacology & Therapeutics Dept.
1974-1976	Cancer Research Scientist II	Grace Cancer Drug Center
1976-1977	Senior Cancer Research Scientist	Roswell Park Institute
1977-1980	Cancer Research Scientist IV	Buffalo, NY 14263
1981-1993	Cancer Research Scientist V	
1993-2000	Cancer Research Scientist VI	
2000-present	Member	

Honors and Professional Memberships

1968-1972	Student Trainee – NIH	University of Rochester,
1975-1982	Assistant Research Professor	School of Medicine Program of Molecular
1982-1985	Director Graduate Program	Pharmacology & Cancer Therapeutics
1982-1987	Associate Research Professor	State University of New York, Buffalo, NY
1987-present	Research Professor	
1987-1990	NCI DCT Review Committee for Disease-Oriented Screening Program	
1990-1994	American Cancer Society Study Section, Biochemistry and Endocrinolog;	
1994-	NIH/NCI-SBIR/STTR Grant Review Committee	
1999-	Co-Director, RPCI Core Drug Development Resource	
1999-	California Breast Cancer Program Review Committee	
2002-	Roswell Park Scientific Review Committee	
2003-	Editorial Board-*Molecular Cancer Therapeutics*	
2005	Member of DPT Review Panel for NCI 60 Screening Program	

Exhibit 1 – Page 1

Selected peer-reviewed publications (in chronological order). Do not include publications submitted or in preparation. (Selected from over 150 peer-reviewed publications)

Porter CW, Bernacki RJ (1975) Ultrastructural evidence for ectoglycoslytransferases systems Nature 256: 648-50.

Bernacki RJ, Kim U (1977) Concomitant elevations in serum sialyltransferase activity and sialic acid content in rats with metastasizing mammary tumors. Science 195: 577-80.

Bernacki, R.J., Niedbala, M.J. and Korytnyk, W. (1985). Glycosidases in Cancer and Invasion. Cancer Metastasis Reviews 4:81-102.

Hong, C.T., Bernacki, R.J., Rustum, Y., Hui, S. and West, C.R. (1990). Formulation, stability and antitumor activity of arabinofuranosylcytosine conjugate of thioether phospholipid. Cancer Res. 50, 4401-4406.

Bernacki, R.J., Bergeron, R.J. and Porter, C.W. (1992). Antitumor activity of N,N'-Bis(ethyl)spermine homologues against human MALME-3 melanoma xenografts. Cancer Res. 52:2424-2430.

Skrincosky, D.M., Allen, H.J. and Bernacki, R.J. (1993). Galaptin mediated adhesion of human ovarian carcinoma A121 cells. Cancer Res. 53:2667-2675.

Pendyala L, Kidani Y, Perez R, Wilkes J, Bernacki RJ, Creaven PJ (1995) Cytotoxicity, cellular accumulation and DNA binding of oxaliplatin isomers. Cancer Lett. 97: 177-84.

Ojima, I., Park, Y-H., Sun, C-M., Fenoglio, I., Appendino, G., Pera, P. and Bernacki, R.J. (1994). Structure-activity relationships of new taxoids derived from 14-hydroxy-10-deacetylbaccatin III. J. Med. Chem. 37:1408-1410.

Woynarowska, B., Skrincosky, D.M., Haag, A., Sharma, M. and Bernacki, R.J. (1994). Inhibition of lectin mediated ovarian tumor cell adhesion by sugar analogs. J. Biol. Chem.269 (36):22797-22803.

Bernacki, R.J., Oberman, E.J., Seweryniak, K.E., Atwood, A., Bergeron, R.J. and Porter, C.W. (1995). Preclinical antitumor efficacy of the polyamine analog, N1,N11-diethylnorspermine, administered by multiple injection or continuous infusion. Clin Cancer Res. 1:847-857.

Sharma, A., Straubinger, R.M., Ojima, I. and Bernacki, R.J. (1995). Antitumor efficacy of taxane liposomes on a human ovarian tumor xenograft in nude athymic mice. J. Pharma. Sci. 84:1400-1404.

Ojima, I., Park, Y.H., Fenoglio, I., Duclos, O., Sun, C.-M., Kuduk, S.D., Zucco, M., Appendino, G., Pera, P., Veith, J.M., Bernacki, R.J., Bissery, M.-C., Combeau, C., Vrignaud, P., Riou, J.F. and Lavelle, F. (1995) Syntheses and Structure-Activity Relationships of New Taxoids. in: Taxane Anticancer Agents: Basic Science and Current Status., G.Geort, T. Chen, I. Ojima, and D.M. Vya (Eds.), ACS Symp. Series 583; The American Chemical Society, Washington, DC, Chapter 19, pp. 262-275.

Woynarowska, B., Dimitroff, C.J., Sharma, M., Matta, K., and Bernacki, R.J. (1996). Inhibition of human HT-29 colon carcinoma cell adhesion by an analog of 4-fluoro-glucosamine. Glycoconjugate J. 13:663-674.

Ojima, I., Slater, J.C., Michaud, E., Kuduk, S.D., Bounaud, P.Y., Vrignaud, P., Bissery, M.C., Veith, J.M., Pera, P., and Bernacki, R.J. (1996). Syntheses and structure-activity relationships of the second-generation antitumor taxoids-exceptional activity against drug-resistant cancer cells. J. Med. Chem. 39:3389-3896.

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Ojima, I., Kuduk, S.D., Slater, J.C., Gimi, R.H., Sun, C.-M., Chakravarty, S., Ourevich, M., Abouabdellah, A., Bonnet-Delpon, D., Begue, J.-P., Veith, J.M., Pera, P. and Bernacki, R.J. (1996). Synthesis, Biological Activity, and Conformational Analysis of Fluorine-Containing Taxoids. In: Biomedical Frontiers of Fluorine Chemistry, I. Ojima, J. McCarthy, and J.T. Welch (eds.), ACS Symp. Series 639, Chapter 17, pp. 228-243.

Ojima, I., Slater, J.C., Kuduk, S.D., Takeuchi, C.S., Gimi, R.H., Sun, C.-M., Park, Y.H., Pera, P., Veith, J.M., and Bernacki, R.J. (1997). Syntheses and structure-Activity relationships of taxoids derived from 14β-Hydroxy-10-deacetylbaccatin III. J. Med. Chem. 40:267-278.

Ojima, I., Kuduk, S.D., Pera, P., Veith, J.M., and Bernacki, R.J. (1997). Synthesis of and structure-activity relationships of non-aromatic taxoids. Effects of alkyl and alkenyl ester groups on cytotoxicity. J. Med. Chem. 40:279-285.

Ojima, I., Slater, J.C., Pera, P., Veith, J.M., Abouabdellah, A., Begue, J.-P., and Bernacki, R.J. (1997). Synthesis and biological activity of novel 3'-trifluoromethyl taxoids. Bioorg. Med. Chem. Lett. 7:133-138.

Sharma, A., Mayhew, E., Bolczak, L., Cavanaugh, C., Harmon, P., Janoff, A., and Bernacki, R.J., (1997). Activity of paclitaxel liposome formulations against human ovarian tumor xenografts. Int. J. Cancer, 71:103-107.

Sharma, A., and Bernacki, R.J. (1997). Commentary: Ovarian cancer patients with high CA-125 but no symptoms -- should antiangiogenic treatments be considered? Oncol. Res. 9:53-54,1997.

Sharma, A., Glaves-Rapp, D., Porter, C.W., Raghavan, D. and Bernacki, R.J. (1997). Antitumor efficacy of N1,N11-diethylnorspermine against a human bladder tumor xenograft. Clin. Cancer Res. 3: 1239-1244,1997.

Dimitroff, C. J., Sharma, A., and Bernacki, R.J.(1997) Cancer Metastasis: A search for therapeutic inhibition. Cancer Invest. 16(4): 279-290.

Kramer, D.L., Fogel-Petrovic, M., Diegelman, P., Cooley, J., Bernacki, R.J., McManus, J.S., Bergeron, R.J., and Porter, C.W. (1997). Effects of novel spermine analogs on cell cycle progression and apoptosis in MALME-3M human melanoma cells. Cancer Research 57:5521-5527.

Mi Z, Kramer DL, Mill JT, Bergeron RJ, Bernacki RJ, Porter CW (1998) Human prostatic carcinoma cell lines display altered regulation of polyamine transport in response to polyamine analogs and inhibitors. Prostate 34: 51-60.

Ojima I, Bounaud PY, Takeuchi C, Pera P, Bernacki RJ (1998) New taxanes as highly efficient reversal agents for multidrug resistance in cancer cells. Bioorg Med Chem Lett 8: 189-94.

Ojima I, Wang T, Miller ML, Lin S, Borella CP, Geng X, Pera P, Bernacki RJ (1999) Synthesis and structure-activity relationships of new second-generation taxoids. Bioorg Med Chem Lett 9: 3423-8.

Dimitroff, C.J., Pera, P., Dall'Olio, F., Matta, K.L., Chandrasedaran, E.V., Lau, J.T., and Bernacki, R.J. (1999) Cell surface N-acetylneuraminic acid α2,3 galactoside-dependent intercellular adhesion of human colon cancer cells. BBRC 256: 631-636.

Dimitroff, C., Klohs, W., Sharma, A., Oh, L., Pera, P., Driscoll, D., Veith, J., Steinkampf, R., Schroeder, M., Kluchko, S., Sumlin, A., Henderson, B., Dougherty, T.J., and Bernacki, R.J. (1999). Antiangiogenic activity of selected receptor tyrosine kinases, PD166285 and PD173074: Implications for combination treatment with photodynamic therapy. Investigational New Drugs 17: 121-135.

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Bernacki RJ, Pera P, Gambacorta P, Brun Y, Greco WR (2000) In vitro antitumor activity of 9-nitro-camptothecin as a single agent and in combination with other antitumor drugs. Ann NY Acad. Sci. 922:293-7.

Ojima I, Lin S, Slater JC, Wang T, Pera P, Bernacki RJ, Ferlini C, Scambia G (2000) Syntheses and biological activity of C-3'-difluoromethyl-taxoids. Bioorg Med Chem 8: 1619-28.

Vredenburg, M.R., Ojima, I., Veith, J., Pera, P., Kee, K., Cabral, F., Sharma, A., Kanter, P., and Bernacki, R.J. (2001) Orally-active taxanes which modulate P-glycoprotein, overcoming resistance in colon and breast carcinomas. J. Natl. Cancer Inst. 93: 1234-1245.

Gruol, D.J., Bernd, J., Phippard, A.E., Ojima, I., and Bernacki, R.J. (2001) The use of a novel taxane-based P-glycoprotein inhibitor to identify mutations that alter the interaction of the protein with paclitaxel. Mol. Pharm. 60: 104-113.

Chibale, K., Ojima, I., Haupt, H., Geng, X., Pera, P., and Bernacki, R.J. (2001) Modulation of human mammary cell sensitivity to paclitaxel by new quinoline sulfonamides. Bioorg. Med. Chem. Lett. 11: 2457-2460.

Ojima, I., Kuduk, S.D., Chakravarty, J.C., Lin, S., Wang, T., Geng, X., Miller, M.L., Bounaud, P.-Y., Michaud, E., Park, Y. H., Sun, C.-M., Slater, Y.C., Inoue, T., Borella, C.P., Walsh, J.J., Bernacki, R.J., Pera, P., Veith, J., Bombardelli, E., Riva, A., Rao, S., He, L., Orr, G.A., Horwitz, S. B., Danishefsky, S.J., Scambia, G., And Ferlini, C. (2001) New generation taxoids and hybrids of microtubule-stabilizing anticancer agents. In: Anticancer Agents: Frontiers in Cancer Chemotherapy, ACS Symp. Ser. 796, Am. Chem. Soc., Washington, D.C., Chapter 4:pp 59-80.

Leontieva, O. V., Preobrazhenskaya, M.N., and Bernacki, R.J. (2002) Partial circumvention of P-glycoprotein-mediated multidrug resistance by doxorubicin 14-O-hemiadipate. Investigational New Drugs, 20: 35-48.

Marasco, CJ Jr., Kramer, DL, Miller, J, Porter, CW, Bacchi, CJ, Rattendi, D, Kucera, L, Iyer, N, Bernacki, R, Pera, P, and Sufrin, JR. (2002). Synthesis and evaluation of analogues of 5'-([(Z)-4-amino-2-butenyl] methylamino)-5'-deoxyadenosine as inhibitors of tumor cell growth, trypanosomal growth, and HIV-1 infectivity. J. Med. Chem. 45: 5112-22.

Ojima, I, Geng, X, Lin S, Pera P, Bernacki, RJ (2002) Design, synthesis and biological activity of novel C2-C3' N-linked macrocyclic taxoids. Bioorg. Med. Chem Lett 12: 349-52.

Dimitroff CJ, Bernacki RJ, Sackstein, R. (2003) Glycosylation-dependent inhibition of cutaneous lymphocyte-associated antigen expression: implications in modulating lymphocyte migration to skin. Blood 101: 602-10.

Wu M, Mazurchuk R, Chaudhary, ND, Spernyak J, Veith J, Pera P, Greco W, Hoffman RM, Kobayashi T, Bernacki RJ (2003) High resolution magnetic resonance imaging of efficacy of cytosine analog CS-682 in a liver-metastasis nude mouse model. Cancer Res. 63: 2477-2482.

Brooks, T., Minderman, H., O'Louglin, K., Pera, P., Ojima, I., Baer, M., and Bernacki, R.J. (2003) Taxane-based reversal agents modulate drug resistance mediated be P-glycoprotein, multidrug resistance protein and breast cancer resistance protein. Molecular Cancer Therapeutics 2: 1195-1205.

Ling, X., Bernacki, R.J., Brattain, M.G. and Li, F (2004) Induction of survivin expression by taxol (paclitaxel) is an early event which is independent of taxol-mediated G2/M arrest. J. Biol. Chem.279:15196-203.

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Minderman, H., Brooks, T., O"Loughlin, K.L., Ojima, I., Bernacki, R.J., and Baer, M. (2004) Modulation of ATP-binding-cassette transport proteins by the taxane derivatives BAY 59-8862 and tRA96023. Cancer Chem. Pharm.53:363-369.

Brooks, T.A., Kennedy, D.R., Gruol, D.J., Ojima, I., Baer, M., and Bernacki, R.J. (2004) Structure-activity analysis of taxane-based broad-spectrum multidrug resistance modulators. Anticancer Res.24: 409-416.

Appendino, G., Bettoni, P., Noncovich, A., Sterner, O., Bombardelli, E., Pera, P., and Bernacki, R.J., (2004) Structure-activity relationships of ring C-secotaxoids. 1. Acylative modifications. J. Nat. Prod. 67: 184-8.

Ramnath, N., Hamm, J., Schwartz, G., Holden, S., Eckhardt, S.G., Vredenburg, M.R., Bernacki, R.J., Kaidbey, J., Lathia, C., Kanter, P. and Creaven, P.J. (2005) A phase I and pharmacokinetic study of BAY 59-8862: A novel taxane. Oncology 67: 123-129.

Ojima, I., Borella, C.P., Wu, X., Bounaud, P-Y, Fumerao, C., Sturm, M., Miller, M.L., Chakravarty, S., Chen, J., Huang, Q., Pera, P., Brooks, T.A., Baer, M.R., and Bernacki, R.J. (2005) Design, synthesis and structure-activity relationships of novel taxane-based multidrug resistance reversal agents. J. Med. Chem. (In Press).

Bouvet, M., Sperynak, J., Katz, MH, Mazurchuk, RV, , Takimoto, S., Bernacki, R, Rustum, YM, Moossa, AR and Hoffman, RM. (2005) High correlation of whole-body red fluorescent protein imaging and magnetic resonance imaging on an orthotopic model of pancreatic cancer. Cancer Res. 65: 9829-33.

Marasco, C.J., Pera P.J., Spiess, A.J., Bernacki, R., Sufrin, J.R. Improved synthesis of β-D-6 methylpurine riboside and antitumor effects of the β-D- and α-D-anomers. (2005) Molecules 10:1015-1020.

Kuznetsova, L, Chen, J, Sun, L, Wu, X, Pepe, A, Veith, JM, Pera, P, Bernacki, RJ, and Ojima, I (2006) Syntheses and evaluation of novel fatty acid-second-generation taxoid conjugates as promising anticancer agents. Bioorganic & Medicinal Chemistry Letters 16: 974-977.

Dennis, C.G., Greco, W.R., Brun, Y., Youn, R., Slocum, H.K., Bernacki, R.J., Lewis, R., Wiederhold, N., Holland, S. M., Petraitiene, R., Walsh, T.J. and Segal, B.H. (2006) Combination amphotericin B and micafungin prolong survival in experimental aspergillosis in the p47$^{phox-/-}$ mouse model of chronic granulomatous disease. (Antimicrobial Agents and Chemotherapy 50, 422-427.).

Brooks TA, O'Loughlin, KL, Minerman, H, Bundy, BN, Ford, LA, Preibe, W., Vredenburg, MR, Bernacki, RJ, and Baer, MR. (2007). The 4"-O-benzylated doxorubicin analog WP744 overcomes resistance mediated by P-glycoprotein, multidrug resistance protein and breast cancer resistance protein in cell lines and acute myeloid leukemia cells. Invest. New Drugs 25: 115-122

C. Research Support. List selected ongoing or completed (during the last three years) research projects (federal and non-federal support). Begin with the projects that are most relevant to the research proposed in this application. Briefly indicate the overall

Exhibit 1 – Page 5

goals of the projects and your role (e.g. PI, Co-Investigator, Consultant) in the research project. Do not list award amounts or percent effort in projects.

Ongoing:

1. Consortium grant 1 RO1 CA118213-01 entitled " Pharmacokinetic strategies to optimize IP chemotherapy", PI Dr. Joseph Balthasar, University at Buffalo, School of Pharmacy, Department of Pharmaceutical Sciences, PI- Dr. Ralph J. Bernacki, Roswell Park Cancer Institute, Department of Pharmacology & Therapeutics. 07/01/06-06/30/10.

The research proposes to investigate inverse targeting strategy that utilizes adjuvant agents such as anti-drug antibodies and anti-angiogenic therapy to create regio-specific alterations in drug disposition to enhance drug targeting (IP) intraperitoneal ovarian cancer.

2. Consortium grant 1RO1 GM074776-01 entitled " Natural product leads for drug development" PI Dr. K. Parker, Department of Chemistry, SUNY Stony Brook; PI Dr. R Bernacki, Department of Pharmacology and Therapeutics, Roswell Park Cancer Institute. 12/1/06-11/30/10.

The research proposes to synthesize and evaluate natural product SNF 4435 C and D and related structures as potential immunosuppressives and/or multidrug resistance modulators.

Completed:

1)   NIH RO1- CA73872-03 "Development of new semisynthetic taxanes." 7/1/99-6/30/04

            P.I. R.J. Bernacki, Ph.D.

            The goal of this program is the design and development of new semisynthetic taxanes having the ability to overcome multidrug resistance.

Exhibit 1 – Page 6

Cytotoxicity of High Energy Compounds

Against HT29 Cancer Cells (IC50)

Chemical compounds	Symbol	IC50 (mM)	Solubility
Cyclotetramethylene tetranitramine	HMX	0.14	++
Cyclonite (Hexahydro-1,3,5-trinitro-s-triazine)	RDX	0.70	++++
CL-20	CL-20	0.07	++++
1-Acetylhexahydro-3,5-dinitro-1,3,5-triazine	TEX	0.088	++
Nitrotriazolone	NTO	4.5	++++
Ammonium Dinitriamide	ADN	4.46	+++++
2,3,5,6-tetrahydroxy1-1,4-diformyl- piperazine	THDFP	0.63	++++
3,4'-diaminofurazan	DAF	0.56	+++++
4,4'-diamino-3,3'azoxyfurazan	DAAOF	0.011	+
TNAZ	TNAZ	0.022	+++
1-tert-butyl-3,3-dinitroazetidium trifluoroacetate	TBDNAZ	0.041	++++
1-Fumary1-3,3-dinitroazetidine	FDNAZ	0.029	+++
1-Succiny1-3,3-dinitroazetidine	SDNAZ	0.087	++
N-Acetyl-3,3-dinitroazetidine	ADNAZ	0.04	++++
1-Acetylbromo-3,3-dinitroazetidine	ABDNAZ	0.001	++++
1-Trifluoroacety1-3,3-dinitroazetidine	TFADNAZ	0.092	+
1-Azidoacety1-3,3-dinitroazetidine	AzADNAZ	0.07	++
1-Chloroacety1-3,3-dinitroazetidine	CADNAZ	0.011	+++
1-Iodoacety1-3,3-dinitroazetidine	IADNAZ	0.0014	++
3-nitratomethyl-3-methyl oxetane	NMMO	4.5	+++
3,3-bis-nitratomethyloxetane	BNMO	2.2	+++
Diglycerol tetranitrate	DGTN	0.35	+++
Triethylene glycol dinitrate	TEGDN	1.33	+++++
Glycidyl nitrate	GLYN	0.13	+++++
1,1,1-trimethylolethane trinitrate	TMETN	0.089	+++++
Pentaerythrite tetranitrate	PETN	5.45	+++
N-butyl-2-nitratoethyl-nitramine	BuN ENA	0.13	+++++
Cisplatin	**CDDP**	**0.004**	**++++**

Exhibit 2

Cytotoxicity of High Energy Compounds
Against HT29 Cancer Cells (IC50 <1.0 mM)

Chemical compounds	Symbol	IC50 (mM)	Solubility
Cyclotetramethylene tetranitramine	HMX	0.14	++
Cyclonite (Hexahydro-1,3,5-trinitro-s-triazine)	RDX	0.70	++++
CL-20	CL-20	0.07	++++
1-Acetylhexahydro-3,5-dinitro-1,3,5-triazine	TEX	0.088	++
2,3,5,6-tetrahydroxy1-1,4-diformyl- piperazine	THDFP	0.63	++++
3,4'-diaminofurazan	DAF	0.56	+++++
4,4'- damino-3,3'azoxyfurazan	DAFAOF	0.011	+
TNAZ	TNAZ	0.022	+++
1-tert-I2uty1-3,3-dinitroazetidium trifluoroacetate	TBDNAZ	0.041	++++
1-Fumary1-3,3-dinitroazetidine	FDNAZ	0.029	+++
1-Sucpiny1-3,3-dinitroazetidine	SDNAZ	0.087	++
N-Acetyl-3,3-dinitroazetidine	ADNAZ	0.04	++++
1-Acetylbromo-3,3-dinitroazetidine	ABDNAZ	0.001	++++
1-Trifluoroacety1-3,3-dinitroazetidine	TFADNAZ	0.092	+
1-Azidoacety1-3,3-dinitroazetidine	AzADNAZ	0.07	++
1-Chloroacety1-3,3-dinitroazetidine	CADNAZ	0.011	+++
1-Iodoacety1-3,3-dinitroazetidine	IADNAZ	0.0014	++
Diglycerol tetranitrate	DGTN	0.35	+++
Glycidyl nitrate	GLYN	0.13	+++++
1,1,1-trimethylolethane trinitrate	TMETN	0.089	+++++
N-butyl-2-nitratoethyl-nitramine	BuNENA	0.13	+++++
Cisplatin	CDDP	0.004	++++

Exhibit 3

In-vitro Activity of Dinitroazetidine Compounds Compared to Cisplatin in HT29 cells
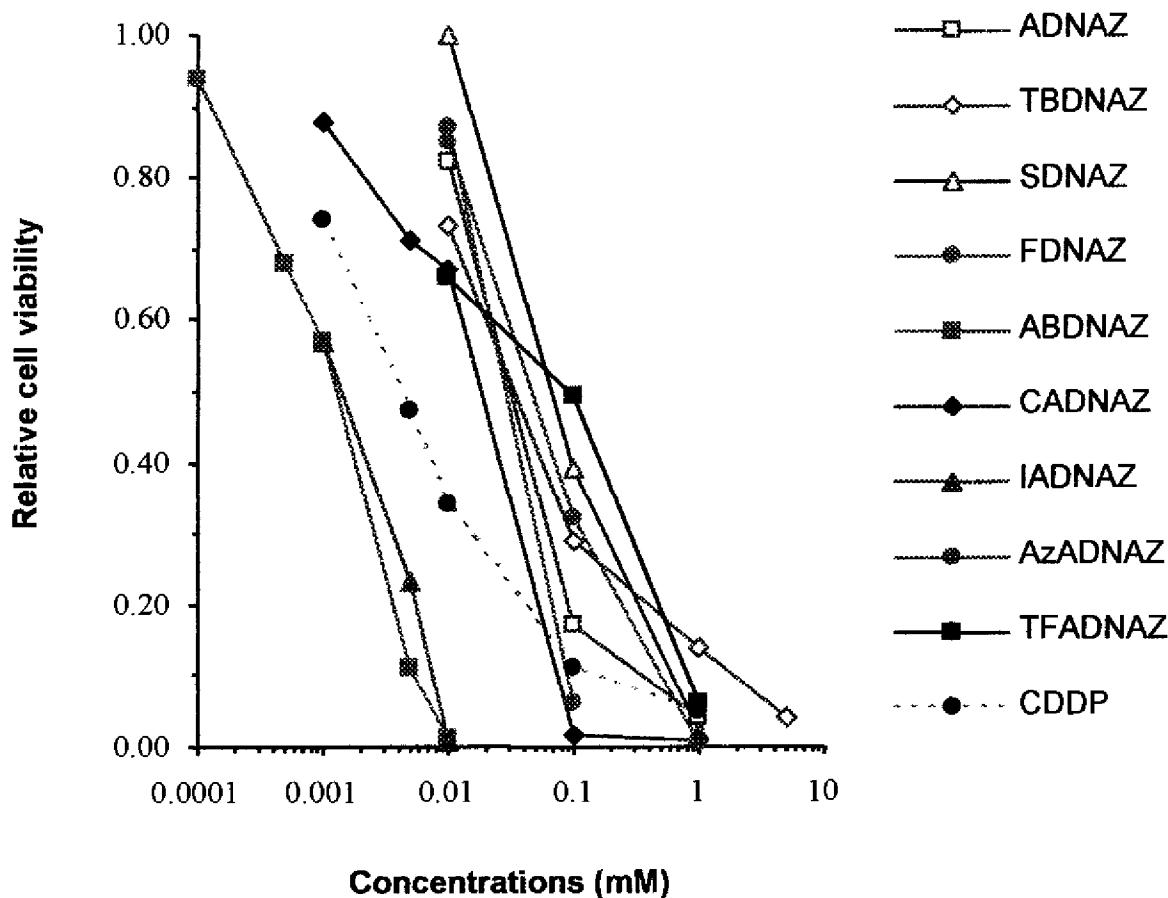

Exhibit 4

ABDNAZ Is Effective Across Multiple Cell Lines
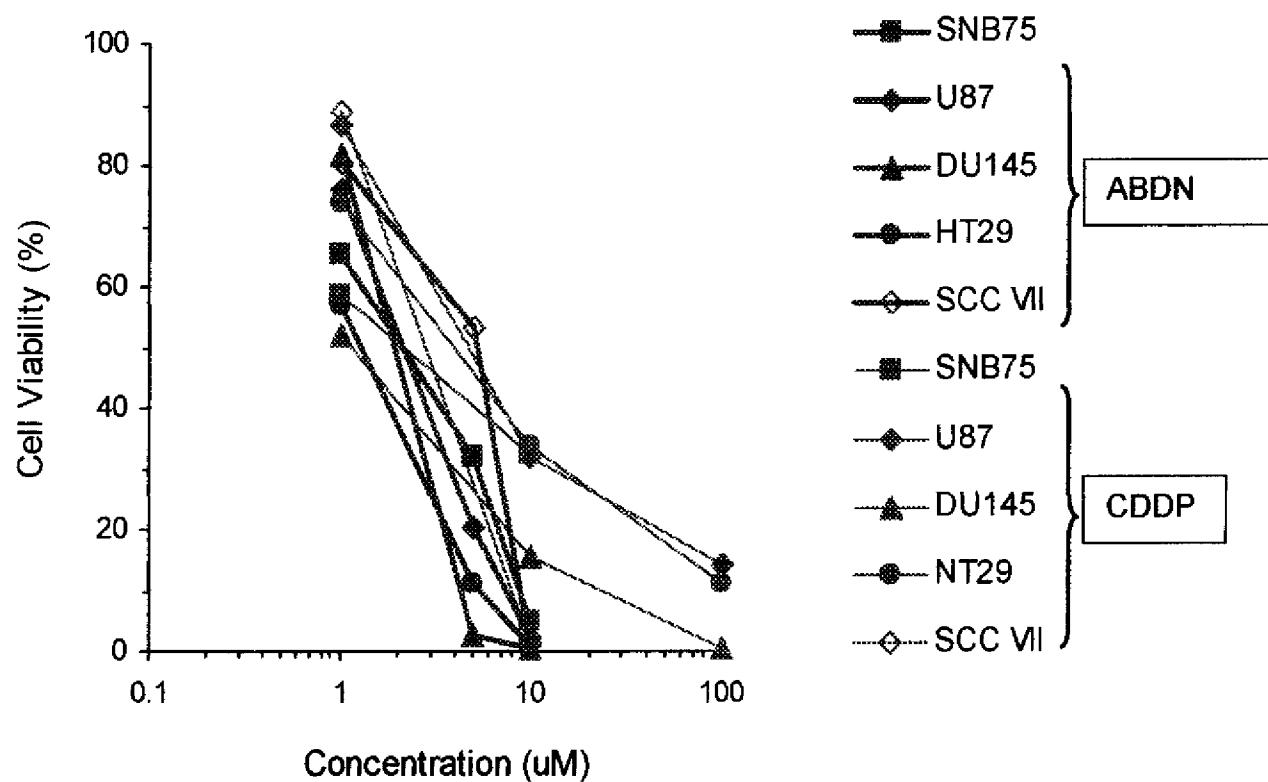

Exhibit 5

[00128]   The components of the kit may be provided in one or more liquid solutions, preferably, an aqueous solution, more preferably, a sterile aqueous solution. The components of the kit may also be provided as solids, which may be converted into liquids by addition of suitable solvents, which are preferably provided in another distinct container.

[00129]   The container of a therapeutic kit may be a vial, test tube, flask, bottle, syringe, or any other means of enclosing a solid or liquid. Usually, when there is more than one component, the kit will contain a second vial or other container, which allows for separate dosing. The kit may also contain another container for a pharmaceutically acceptable liquid.

[00130]   Preferably, a therapeutic kit will contain apparatus (e.g., one or more needles, syringes, eye droppers, pipette, etc.), which enables administration of the components of the kit.

EXAMPLES

[00131]   The invention is further defined by reference to the following examples, which describe in detail, preparation of compounds and methods for assaying for biological activity. It will be apparent to those skilled in the art that many modifications, both to materials and methods, may be practiced without departing from the scope.

Example 1: Production of ROS in Tumor Cells by ABDNAZ and Irradiation

[00132]   Human colon cancer cell line HT29 cells and murine squamous cell carcinoma cell line SCC VII cells were grown in 96-well plate overnight at 37°C and then a fluorescent probe 2'7'-dichlorofluorescin diacetate (DCFH-DA) was added at a concentration of 20 μM for 1 hour and then washed out. ABDNAZ was added in the growth media at concentrations of 1 μM, 10 μM or 100 μM. The green fluorescence was observed under a fluorescence microscope and measured using a microplate spectrofluorometer with an excitation at 488 nm and an emission at 525 nm. For cells that were treated with both ABDNAZ and

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irradiation, the plates were irradiated immediately after addition of ABDNAZ
using a ^{137}Cs source.

[00133]   Figure 1 shows the production of reactive oxygen species (ROS) in
HT29 cells and SCC VII cells after exposure to ABDNAZ. The production of
ROS was dose, time and cell line dependent. The ROS production in HT29 cells
was gradually increased over time and peaked at 24 hours. For SCC VII cells, the
production of ROS was peaked 2 hours after addition of ABDNAZ, and the levels
of ROS were significantly higher than that induced in HT29 cells.

[00134]   Figures 2 and 3 illustrate the ROS production in HT29 cells and SCC
VII cells treated with ABDNAZ and radiation. Combined treatment of ABDNAZ
and radiation synergistically induced intracellular ROS generation in HT29 cells
and SCC VII cells, as compared with each modality alone.

Example 2: Inhibition of Proliferation of HL60 cells by ABDNAZ
[00135]   HL60 cell line which is an acute promyelocytic leukemia cell line was
stably transfected with bcl-2 oncogene (HL60 bcl-2 cells). The HL60 neo cells
were used as a control (HL60 neo). Cells were grown in RPMI1640 media in the
presence of ABDNAZ at a concentration of 1 uM, 2 uM or 5 uM. The number of
viable cells was counted daily for 10 days. The cell growth curves were shown in
Figure 4 which demonstrates that ABDNAZ inhibited cell growth in a dose-
dependent manner. A dose of 5uM of ABDNAZ inhibited cell growth by > 95%
and HL60 bcl-2 cells were as sensitive as neo cells to ABDNAZ.

Example 3: Induction of Apoptosis of HL 60 cells by ABDNAZ
[00136]   Cells, prepared and grown as described in Example 2, *supra*, were
collected at 8, 24, 48, and 72 hours after addition of ABDNAZ, and analyzed
using FACS. Figure 5 illustrates the percent of apoptosis vs. time in the presence
of ABDNAZ. As can be seen in Figures 5, 6 and 7, ABDNAZ induced a very high
level of apoptotic cell death in both HL60 neo and bcl-2 cells in a dose-dependent
manner. ABDNAZ at 5 uM induced 95% and 78% apoptosis at 48 hours for neo
and bcl-2 cells, respectively. At 2 uM, ABDNAZ produced apoptotic cell death
that was very similar in HL60 neo and bcl-2 cells with peaks of ~40% at 8 hours.

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Figures 6 and 7 illustrates the detailed histograms of FACS analysis for HL60 neo cells and HL60 bcl-2 cells, respectively.

Example 4: Inhibition of bcl-2 Oncogene Expression by ABDNAZ

[00137]   HL60 cells were treated as described in Example 2, *supra*. Cells were collected at 6 and 24 hours for Western blot analysis. As shown in Figure 8, ABDNAZ at 2 and 5 uM inhibited bcl-2 protein expression in both neo and bcl-2 cells in a dose-dependent manner. The bcl-2 protein in HL60 bcl-2 transfected cells may be cleaved in the presence of 2uM ABDNAZ as indicated by the presence of the lower molecular weight bands after both 6 and 24 hours.

Example 5: Synthesis of ABDNAZ

[00138]   A 25 ml, three-neck, round bottom flask was charged with 7 ml of methylene chloride and 2.50 g (12.3 mmol) of t-BuDNAZ prepared as described in Archibald *et al.*, *Journal of Organic Chemistry*, 1990, 2920. Under nitrogen, 0.16 ml (1.23 mmol) of boron trifluoride etherate was added. After stirring 5 min. at ambient temperature, 0.54 ml (6.15 mol) of bromoacetyl bromide was added. The solution was heated between 50-60°C for 2 h. The darkened reaction mixture was cooled to ambient temperature, diluted with 50 ml methylene chloride, and filtered. The solid was identified as the HBr salt of t-BuDNAZ. The methylene chloride filtrate was washed with two 20 ml portions of water, dried over sodium sulfate, filtered, and evaporated under reduced pressure. The resultant solid was washed with three 20 ml portions of ethyl ether and dried under vacuum to yield 1.24 g (75.2% based on bromoacetyl bromide) of BrADNAZ as a white solid (mp = 124-125°C). ^{1}H NMR (CDCl$_3$): δ 3.76 (s, 2H), 4.88 (br s, 2H), 5.14 (br s, 2H); ^{13}C NMR (CDCl$_3$): δ 165.2, 105.0, 59.72, 57.79, 23.90. Calc. for C$_5$H$_6$BrN$_3$O$_5$: %C 22.41, %H 2.26, %N 15.68; Found: %C 22.61, %H 2.36, %N 15.58. HPLC/MS C-8 reverse phase column with acetonitrile/water mobile phase – m/e 266.95 (100%), 268.95 (98.3%). FT-IR 3014.24 (weak), 1677.66, 1586.30, 1567.65, 1445.55 (NO2), 1367.80, 1338.00, 1251.27 cm^{-1}.

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Example 6: Synthesis of N-(chloroacetyl)-3,3-dinitroazetidine (ClADNAZ)

[00139] A 25 ml, three-neck, round bottom flask was charged with 7 ml of methylene chloride and 2.50 g (12.3 mmol) of t-BuDNAZ. Under nitrogen, 0.16 ml (1.23 mmol) of boron trifluoride etherate was added. After stirring 5 min. at ambient temperature, 0.54 ml (6.15 mol) of chloroacetyl chloride was added. The solution was heated between 50-60°C for 2 h. The darkened reaction mixture was cooled to ambient temperature, diluted with 50 ml methylene chloride and filtered. The solid was identified as the HBr salt of t-BuDNAZ. The methylene chloride filtrate was washed with two 20 ml portions of water, dried over sodium sulfate, filtered, and evaporated under reduced pressure. The resultant solid was washed with three 20 ml portions of ethyl ether and dried under vacuum to afford a white solid (mp: 130-132°C) in 60% yield. CHN for $C_5H_6ClN_3O_5$: Found C: 26.94%, H: 2.53%, N:17.77%; Calculated C: 26.86%, H: 2.71% N: 18.79%. FTIR: 2979(weak), 1690.01, 1577.57, 1438.91(NO_2), 1368.21, 1338.99, 1286.21 cm^{-1}. ^{1}H NMR: (DMSO-d$_6$), δ 5.09(2H), 4.81(2H), 3.77(2H). ^{13}C NMR: (DMSO-d$_6$), δ 168.58, 106.98, 60.39, 50.38. HPLC: >98% pure. Safety Data: ABL Impact: 80 cm; ABL Friction: 800@ 8 ft/sec; TC ESD Unconfined at 50%: 1.10 Joules (mass ignition on bulk test). DSC Onset: 259.56°C.

Example 7: Synthesis of N-Iodoacetyl-3,3-dinitroazetidine(IADNAZ)

[00140] A 100 ml, three neck round bottom flask was charged with 40 mL of anhydrous acetone and 2.01g of BrADNAZ under nitrogen. 1.4 g K_2CO_3 was added followed by the addition of 1.2g sodium iodide. The reaction mixture was allowed to reflux overnight and monitored by proton NMR. The darkened solution was diluted with methylene chloride, the solid was filtered and the filtrate was extracted with 2X 30 mL portions of methylene chloride and water. The organic layer was dried over sodium sulfate and concentrated under vacuum. The solid was purified by flash column chromatography (10% ethyl acetate/hexanes) to yield a white solid (mp 97-100°C) in 80% yield. Analysis for $C_5H_6IN_3O_5$: Found C: 19.67%, H: 1.80%, N: 12.70%. Calculated C: 19.06%, H: 1.92%, N: 13.24%. FTIR: 2980 (weak), 1667.44, 1568.49, 1439.74 (NO_2), 1373.69, 1335.60, 1305.35 cm^{-1}. ^{1}H NMR: (DMSO-d$_6$), δ 5.09(2H), 4.81(2H), 3.77(2H). ^{13}C NMR: (DMSO-d$_6$), δ 168.58, 106.98, 60.39, 50.38. HPLC: >98% pure. Safety Data: ABL Impact: 80 cm; ABL Friction: 800@ 8 ft/sec; TC ESD,

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Unconfined 50% 7.30 Joules(no mass ignition on bulk test); SBAT Onset: 286 °F.
DSC Onset: 253.52 °C.

Example 8: Synthesis of N-Azidoacetyl-3,3-dinitroazetidine(AzADNAZ)

[00141]   A 100 ml, three neck flask was charged with 40 mL of anhydrous
acetone and 2.01g of BrADNAZ under nitrogen. 1.05 g K_2CO_3 was added
followed by the addition of 0.4g sodium azide. The reaction mixture was allowed
to reflux overnight and monitored by proton NMR. The darkened solution was
diluted with methylene chloride and the solid was filtered. The filtrate was
extracted with 2 30 mL portions of methylene chloride and water. The organic
layer was dried over sodium sulfate and concentrated under vacuum. The solid
was purified by flash column chromatography (10% Ethyl acetate/Hexanes) to
yield a white solid (mp 103-104°C) in 80% yield. Analysis for $C_5H_6N_6O_5$: Found
C: 26.84%, H: 2.70%, N: 35.49%. Calculated C: 26.09%, H: 2.63%, N: 34.76%.
FTIR: 2981.60 (weak), 2109.15 (strong), 1678.88, 1598.80, 1571.47, 1463.18
(NO_2), 1446.89, 1332.20, 1275.28 cm⁻¹. ^1H NMR: (DMSO-d_6), δ 5.08(2H),
4.83(2H), 4.02(2H). ^{13}C NMR: (DMSO-d_6), δ 169.098, 107.74, 59.84, 58.16.
HPLC: >99.7% pure. Safety Data: ABL Impact: 64 cm; ABL Friction: 800@ 8
ft/sec; TC ESD, Unconfined 50% < 0.5 Joules(no mass ignition on bulk test);
SBAT Onset: 314 °F.

Example 9: Synthesis of N-Succinyl-3,3-Dinitroazetidine

[00142]   A 100 ml, three neck round bottom flask was charged with 30 mL of
anhydrous dichloromethane and 5.0 grams of tert-butyl-3,3-dinitroazetidine(t-
BDNAZ) under nitrogen. 4.5 grams of succinyl chloride was added followed by
the addition of 0.5 mL of boron trifluoride etherate. The reaction mixture was
heated to 50 °C and monitored by NMR. The reaction mixture was poured slowly
into ice and then filtered. The brown solid was washed with 3X 20 ml. portions
of dichloromethane, dried with sodium sulfate and concentrated under vacuum.
The solid was purified by flash column chromatography (10% ethyl
acetate/hexanes) to yield a pale white solid in 20% yield (mp: 190-192°C).
Analysis for $C_7H_9N_3O_7$: Found C: 33.93%, H: 3.63%, N: 19%. Calculated C:
34.02%; H: 3.67%; N: 17.00. FTIR: 3004.44(weak), 1644.78(strong), 1558.45,

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1472.60, 1450.06, 1423.01, 1369.90, 1338.05, 1310.05, 1260.99 cm^{-1}. ^{1}H NMR:
(DMSO-d$_6$), δ 5.27(2H), 4.85(2H), 2.03(4H). HPLC: >97%. . Safety Data: ABL
Impact: 64 cm; ABL Friction: 800@ 8 ft/sec; TC ESD, Unconfined at 50% < 0.26
Joules (no mass ignition at 8 Joules). DSC Onset: 253.86 °C.

Example 10: Synthesis of N-Fumaryl-3,3-Dinitroazetidine

[00143]   A 100 ml, three neck round bottom flask was charged with 8.69 grams
of tert-butyl-3,3-dinitroazetidine(t-BDNAZ) under nitrogen and 5 mL of furmaryl
chloride was added followed by the addition of 0.5 mL of boron trifluoride
etherate at 0°C for 2 hours. The reaction mixture was monitored by NMR. The
thick paste was washed with methanol and then poured into ice-water. The solid
was filtered and washed with 200 mL of water and dried under vacuum which
afforded a pale yellow solid in 20% yield (mp: 240°C). Analysis for C$_7$H$_7$N$_3$O$_7$:
Found C: 34.9%, H: 3.2%, N: 19.6%. Calculated C: 34.3%; H: 2.9%; N: 17.1.
FTIR: 3082.73(weak), 1664.79(strong), 1577.69, 1430.19 (NO$_2$), 1366.92,
1274.30, 1231.24, 1213.45 cm^{-1}. ^{1}H NMR: (DMSO-d$_6$), δ 5.88(2H), 5.29(2H),
4.90(2H). HPLC:>96%. Safety Data: ABL Friction: 800@ 8 ft/sec, TC ESD
Unconfined at 50%: 1.05 Joules (mass ignition on bulk test).

Example 11: Synthesis of N-Trifluromethyl-3,3-Dinitroazetidine

[00144]   A 100 ml, three neck round bottom flask was charged with 2.28 grams
of tert-butyl-3,3-dinitroazetidine(t-BDNAZ) under nitrogen. 10 mL of
trifluoroacetic anhydride was added followed by the addition of 0.3 mL of boron
trifluoride etherate. The reaction mixture was heated to 50 °C and monitored by
NMR. The reaction was concentrated under vacuum. The residual oil was washed
with water. The residual oil was added to hot hexanes and to afford 460 mg of
white needles (mp: 70-71°C). Analysis for C$_5$H$_4$F$_3$N$_3$O$_5$: Found C: H:, N:
Calculated C: 24.70%, H: 1.66%, N: 17.29%. FTIR: 2991 (weak), 1716, 1683.96,
1591.37, 1576.43 (NO$_2$), 1165.92, 1134.12 cm^{-1}. ^{1}H NMR: (DMSO-d$_6$), δ
5.39(2H), 5.04(2H). HPLC: >96% pure. Safety Data: ABL Friction: 800@ 8ft/sec;
TC ESD Unconfined at 50%: > 8 Joules. DSC Onset: 240.75 °C.s

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Example 12

1) Cytotoxicity of ABDNAZ under normoxic and hypoxic condition of SCC VII tumor cells.

Study design: SCC VII squamous cell carcinoma cells were planted in 60-mm glass dishes at a cell density of 500,000 cells per dish in Waymouth's growth medium supplemented with 15% fetal calf serum. ABDNAZ was dissolved in growth medium and added to the dishes at final concentrations of 0-5 uM. Cells were incubated for 2 hours at 37°C in hypoxic jigs (95% nitrogen and 5% air), or in normal oxygen condition (95% air and 5% CO_2). After 2-hour incubation, cells were trypsinized, counted and plated into 60-mm petri dishes in triplicate for colony formation. After 14 days incubation, cells were stained with crystal violet. Colonies containing more than 50 cells were counted and used to calculate surviving fractions.

Results: Figure 1 shows the dose response of SCC VII cells to ABDNAZ under normoxic and hypoxic condition. ABDNAZ inhibited the clonogenic survival of SCC VII cancer cells in a dose-dependent manner under both hypoxic and normoxic conditions. However, cells under hypoxic condition were more sensitive to ABDNAZ than that in normal condition. The IC50 (the concentration required for 50% inhibition of colony formation) was 0.093 uM for hypoxic cells and 0.63 uM for normoxic cells, respectively, representing 6.8-times potent under hypoxic condition.

Figure 1. Dose-response of ABDNAZ under hypoxic and normoxic condition.

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1-SF/7710698.1
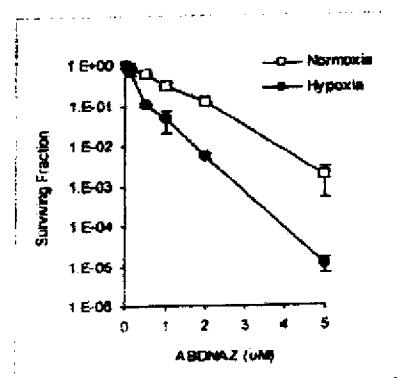
2) Comparison of cytotoxicity of ABDNAZ and Tirapazimine under hypoxic condition.

Study design: SCC VII carcinoma cells were planted in 60-mm glass dishes at a cell density of 500,000 cells per dish in Waymouth's growth medium supplemented with 15% fetal calf serum. ABDNAZ and tirapazimine was dissolved in growth medium and added to the dishes at final concentrations of 0-5 uM. Cells were incubated for 2 hours at 37°C in hypoxic jigs (95% nitrogen and 5% air). After 2-hour incubation, cells were trypsinized, counted and plated into 60-mm petri dishes in triplicate and incubated at 37°C for 14 days. Then dishes were stained with crystal violet. Colonies containing more than 50 cells were counted and used to calculate surviving fractions.

Results: As shown in Figure 2 below, both ABDNAZ and tirapazimine inhibited the clonogenic survival in a dose-dependent manner. However, the survival curve of ABDNAZ was shifted downward dramatically compared to the survival curve of tirapazimine, indicating that ABDNAZ is more potent than tirapazimine in inhibiting the clonogenic formation of SCC VII cells under hypoxic condition. At 5uM, the survivals were 0.0006 for ABDNAZ and 0.1 for tirapazimine, representing a 2-3 log difference in cell survival.

Figure 2. Cytotoxicy of ABDNAZ and tirapazime under hypoxic condition.


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3) Radiosensitization effect of ABDNAZ under hypoxic condition.

Study design: SCC VII cells were planted in 60-mm glass dishes at a cell density of 500,000 cells per dish in Waymouth's growth medium supplemented with 15% fetal calf serum. ABDNAZ was added to the dishes at a final concentrations of 1 uM. Cells were immediately irradiated in hypoxic jigs (95% nitrogen and 5% air) with 0-10 Gy using a ^{137}Cs source with a dose rate of 3 Gy/min. Two hours after radiation, cells were trypsinized, counted and plated into 60-mm petri dishes in triplicate for colony formation. After 14 days incubation, dishes were stained with crystal violet. Colonies containing more than 50 cells were counted and used to calculate surviving fractions.

Results: Figure 3 shows the radiation dose-response survival curves of SCC VII cells with or without ABDNAZ. Radiation alone reduced the survival in a dose-dependent manner. When combined with ABDNAZ, the survival curves decreased significantly. A dose of 1.0 uM ABDNAZ decreased the clonogenic survival by 1 logs at 10Gy.

Figure 3. ABDNAZ radiosensitizes SCC VII cells under hypoxia.
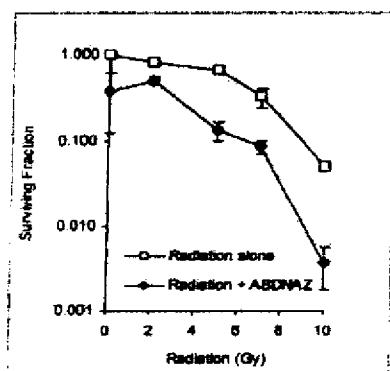
4) Anti-tumor efficacy of ABDNAZ and cisplatin in SCC VII tumor model in mice.

Study design: Mice were subcutaneously implanted in the right flank with 500,000 SCC VII tumor cells in a suspension volume of 50 uL. One tumor per mouse was implanted. When tumors reached an average size of 100 mm^3 (80-150 mm^3),

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animals were treated intraperitoneally with a single dose of ABDNAZ (15mg/kg body weight) or cisplatin (10mg/kg). Six to 8 mice were used in each group. The length, width and height of the tumors were measured with calipers before treatment and three times a week thereafter until the tumor volume reached at least four times (4x) the original pretreatment volume. Tumor volume (in mm³) was calculated according to the formula: tumor volume = $\pi/6$ x length x width x height. Data are expressed as the relative tumor volume to the pretreatment volume on Day 0 and as the mean tumor growth delay time. The tumor growth delay time is the difference between the 4x time of treated tumors compared to that of untreated control tumors. This was calculated for each individual animal treated, and then averaged for each group.

Results: As shown in Figure 4 below, ABDNAZ at a single dose of 15mg/kg and cisplatin at a single dose of 10mg/kg inhibited the tumor growth and produced a 4x tumor growth delay time of 4.5 ± 1.1 and 4.3 ± 0.9 days, respectively. There was no statistically significant differences in tumor growth delay time between ABDNAZ and cisplatin- treated tumors ($P = 0.7$).

Figure 4. Anti-tumor efficacy of ABDNAZ and cisplatin in tumor-bearing mice.
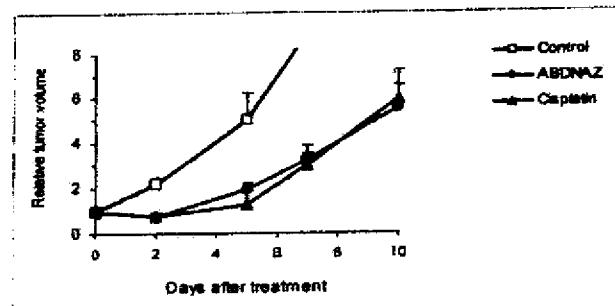
1) Toxicity of ABDNAZ and cisplatin in mice bearing SCC VII tumors.

Mice were subcutaneously implanted in the right flank with 500,000 SCC VII tumor cells. One tumor per mouse was implanted. When tumors reached an average size of 100 mm³ (80-150 mm³), animals were treated intraperitoneally with ABDNAZ (10mg/kg body weight, 3 dose in one week, or 5mg/kg 5 doses in one week) or

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cisplatin (10mg/kg single dose). Six to 8 mice were used in each group. Body weight of the tumor-bearing mice was measured three times weekly. Data are expressed as the relative body weight to the pretreatment weight measured on Day 0.

As shown in Figure below, ABDNAZ at an accumulated dose of 25-30 mg/kg did not decrease the body weight compared to that of untreated control mice. However, cisplatin at a single dose of 10mg/kg caused a decrease in body weight, e.g. a 13% decrease on day 5 after administration of cisplatin compared to untreated control mice.

Figure. Body weight of tumor-bearing mice treated with ABDNAZ and cisplatin.
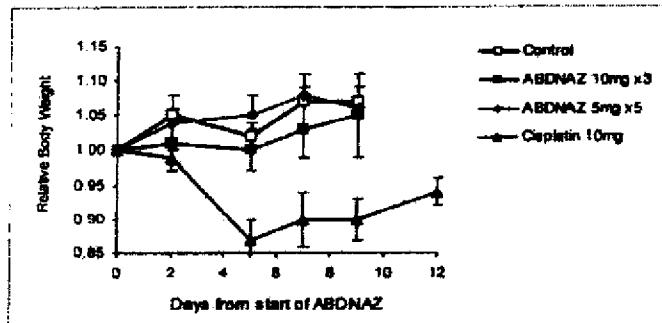
[00145] Finally, it should be noted that there are alternative ways of implementing the present invention. Accordingly, the present embodiments are to be considered as illustrative and not restrictive, and the invention is not to be limited to the details given herein, but may be modified within the scope and equivalents of the appended claims. All publications and patents cited herein are incorporated by reference.

[00146] All references and publications cited herein are incorporated by reference in their entirety.

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WHAT IS CLAIMED IS:

1. A compound of structural Formula (1):

$$R^1 \underset{\underset{R^7}{\overset{|}{N}}}{\overset{\overset{R^2\ \ R^3}{\underset{}{|\ \ |}}}{\text{———}}} \overset{R^4}{\underset{\overset{|}{R^6}}{|_{o}R^5}}$$
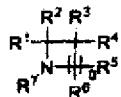
(1)

or salts, solvates or hydrates thereof wherein:

R^1, R^2, R^3 and R^4 are each independently, hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, heteroaryl, substituted heteroaryl, heteroalkyl, substituted heteroalkyl, heteroarylalkyl, substituted heteroarylalkyl, halo, hydroxy or nitro;

each R^5 and R^6 are each independently, hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, heteroaryl, substituted heteroaryl, heteroalkyl, substituted heteroalkyl, heteroarylalkyl, substituted heteroarylalkyl, halo, hydroxy or nitro;

o is 0, 1, 2, 3 or 4;

R^7 is substituted alkyl, substituted arylalkyl, substituted heteroalkyl, substituted heteroaryl, substituted heteroarylalkyl, substituted acyl, substituted alkoxycarbonyl, substituted phosphonyl or substituted sulfonyl;

provided that at least one of R^1, R^2, R^3, R^4, R^5 and R^6 are nitro.

2. The compound of Claim 1 in which at least two of R^1, R^2, R^3, R^4, R^5 and R^6 are nitro.

3. The compound of Claim 1 in which R^1, R^2, R^3 and R^4 are each independently, hydrogen, alkyl or nitro and each R^5 and R^6 are each independently, hydrogen, alkyl or nitro.

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4.   The compound of Claim 1 in which R^7 is substituted alkyl, substituted acyl, substituted alkoxycarbonyl, substituted phosphonyl or substituted sulfonyl.

5.   The compound of Claim 1 in which R^7 is alkyl, acyl, alkoxycarbonyl, phosphonyl or sulfonyl substituted with one or more halogen, $-CF_3$ or $-OS(O)_2R^8$ wherein R^8 is alkyl, substituted alkyl, aryl or substituted aryl.

6.   The compound of Claim 1 in which R^3 and R^4 are nitro.

7.   The compound of Claim 1 in which R^1, R^2, R^3 and R^4 are each independently, hydrogen, alkyl or nitro, each R^5 and R^6 are each independently, hydrogen, alkyl or nitro and R7 is substituted alkyl, substituted acyl, substituted alkoxycarbonyl, substituted phosphonyl or substituted sulfonyl.

8.   The compound of Claim 1 in which R^1, R^2, R^3 and R^4 are each independently, hydrogen, alkyl or nitro, each R^5 and R^6 are each independently, hydrogen, alkyl or nitro and R^7 is alkyl, acyl, alkoxycarbonyl, phosphonyl or sulfonyl substituted with one or more halogen, $-CF_3$ or $-OS(O)_2R^8$ wherein R^8 is alkyl, substituted alkyl, aryl or substituted aryl.

9.   The compound of Claim 1 in which R^1 and R^2 are each independently, hydrogen, alkyl or nitro, R^3 and R^4 are nitro, each R^5 and R^6 are each independently, hydrogen, alkyl or nitro and R^7 is substituted alkyl, substituted acyl, substituted alkoxycarbonyl, substituted phosphonyl or substituted sulfonyl.

10.   The compound of Claim 1 in which R^1 and R^2 are each independently, hydrogen, alkyl or nitro, R^3 and R^4 are nitro, each R^5 and R^6 are each independently, hydrogen, alkyl or nitro and R^7 is alkyl, acyl, alkoxycarbonyl, phosphonyl or sulfonyl substituted with one or more halogen, $-CF_3$ or $-OS(O)_2R^8$ wherein R^8 is alkyl, substituted alkyl, aryl or substituted aryl.

11.   The compound of any of Claims 1-10 in which o is 1.

12.   The compound of Claim 1 in which R^1 and R^2 are each independently, hydrogen, alkyl or nitro, R^3 and R^4 are nitro, each R^5 and R^6 are each independently, hydrogen, alkyl or nitro and R^7 is alkyl or acyl substituted with one or more halogen or $-CF_3$ and o is 1.

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13.   The compound of Claim 1 in which R^1 and R^2 are hydrogen, R^3 and R^4 are nitro, R^5 and R^6 are hydrogen, R^7 is alkyl or acyl substituted with one or more halogen or $-CF_3$ and o is 1.

14.   The compound of Claim 1 having the structure:
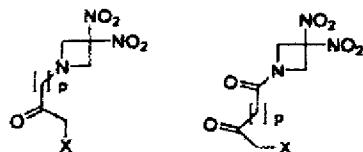
wherein each X is independently -F, -Cl, -Br, -I or $-OS(O)_2R^8$ where R^8 is methyl, CF_3, phenyl or tolyl and each p is independently 1, 2, 3, or 4.

15.   The compound of Claim 1 having the structure:
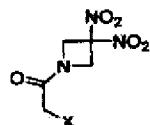
wherein each X is independently -F, -Cl, -Br, -I or $-OS(O)_2R^8$ where R^8 is methyl, CF_3, phenyl or tolyl.

16.   A pharmaceutical composition comprising the compound of Claim 1 and a pharmaceutically acceptable vehicle.

17.   A method for treating or preventing cancer in a patient comprising administering to a patient in need of such treatment or prevention a therapeutically effective amount of the compound of Claim 1.

18.   The method of any one of Claims 16 or 17 in which the cancer is breast cancer, renal cancer, brain cancer, colon cancer, colorectal cancer, prostrate cancer or lung cancer.

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19.   A method for treating tumor cells with a reduced intracellular environment in a patient, comprising administering to the patient in need of treatment a therapeutically effective amount of the compound of Claim 1.

20.   A method for treating or preventing solid tumors in a patient, comprising administering to the patient in need of treatment or prevention a therapeutically effective amount of the compound of Claim 1.

21.   A method for treating or preventing leukemias and lymphomas in a patient, comprising administering to the patient in need of treatment or prevention a therapeutically effective amount of the compound of Claim 1.

22.   A method for treating or preventing inflammation in a patient, comprising administering to the patient in need of treatment or prevention a therapeutically effective amount of the compound of Claim 1.

23.   A method for treating or preventing autoimmune disease in a patient, comprising administering to the patient in need of treatment or prevention a therapeutically effective amount of the compound of Claim 1.

24.   A method for treating or preventing cardiovascular disease in a patient, comprising administering to the patient in need of treatment or prevention a therapeutically effective amount of the compound of Claim 1.

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ABSTRACT OF THE INVENTION

The present invention provides cyclic nitro compound, pharmaceutical compositions
of cyclic nitro compounds and methods of using cyclic nitro compounds and/or
pharmaceutical compositions thereof to treat or prevent diseases or disorders
characterized by abnormal cell proliferation, such as cancer, inflammation,
cardiovascular disease and autoimmune disease.

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Figure 1

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Figure 2

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Figure 3

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1-SF/7710698.1
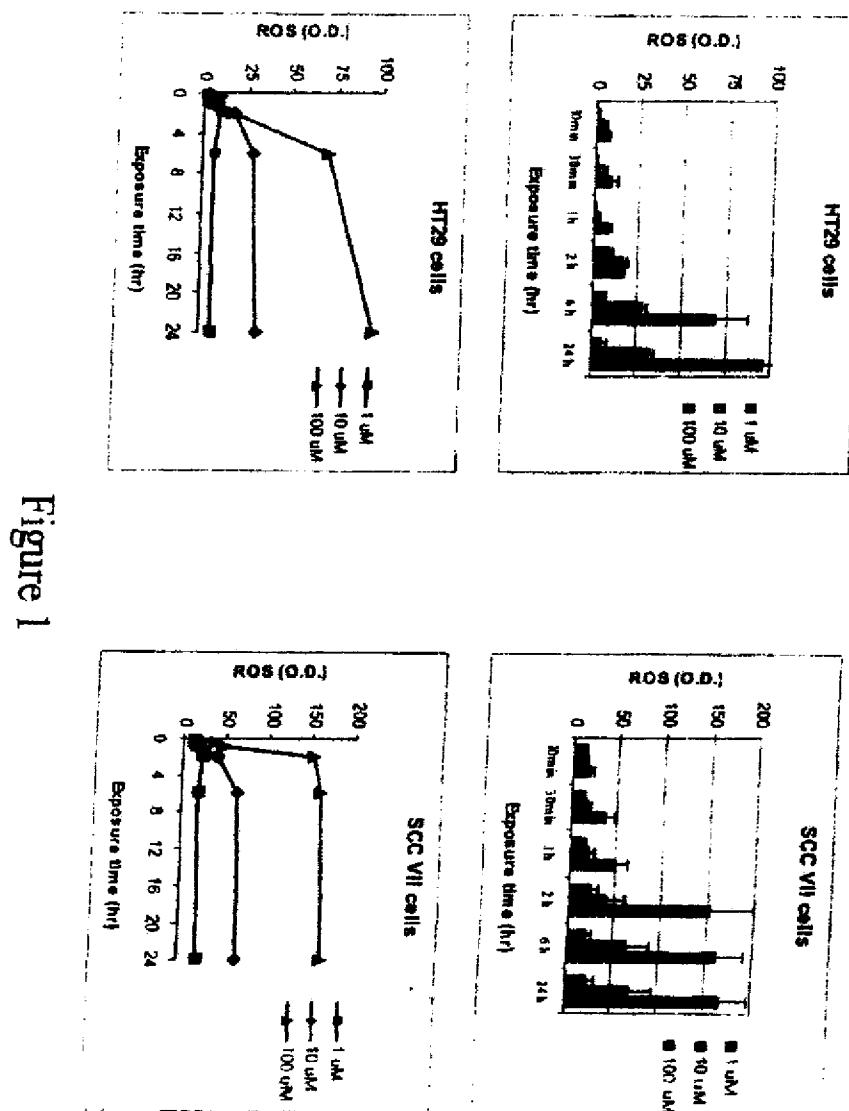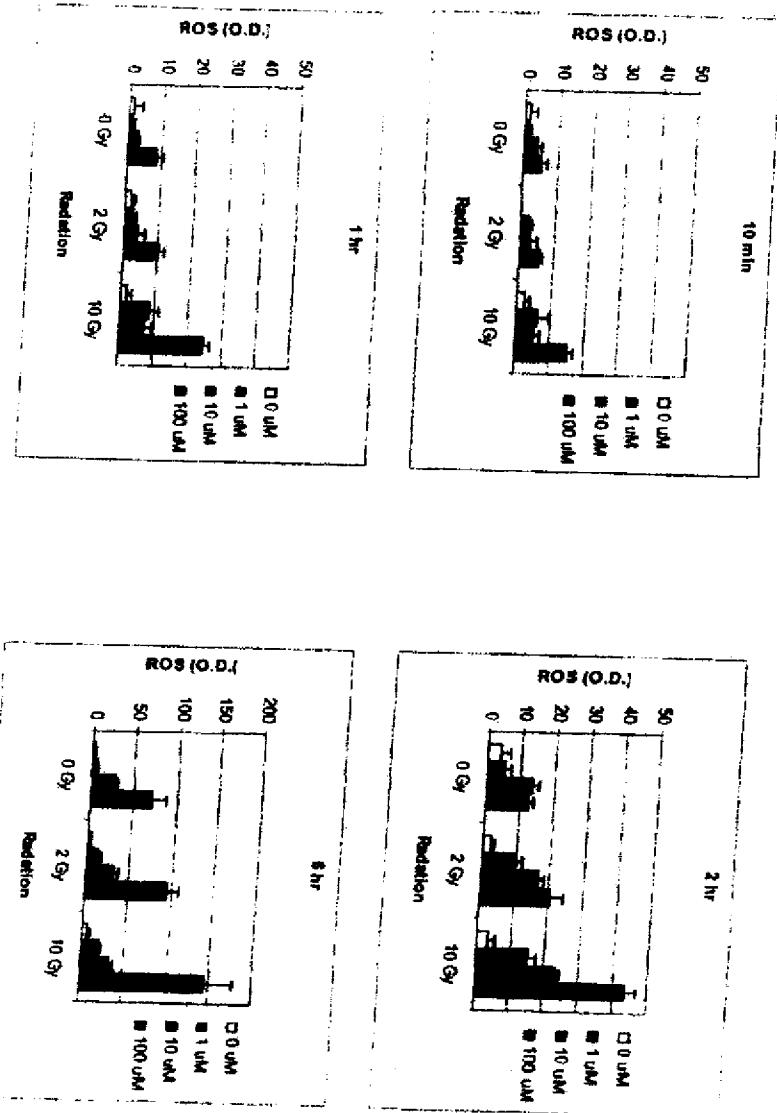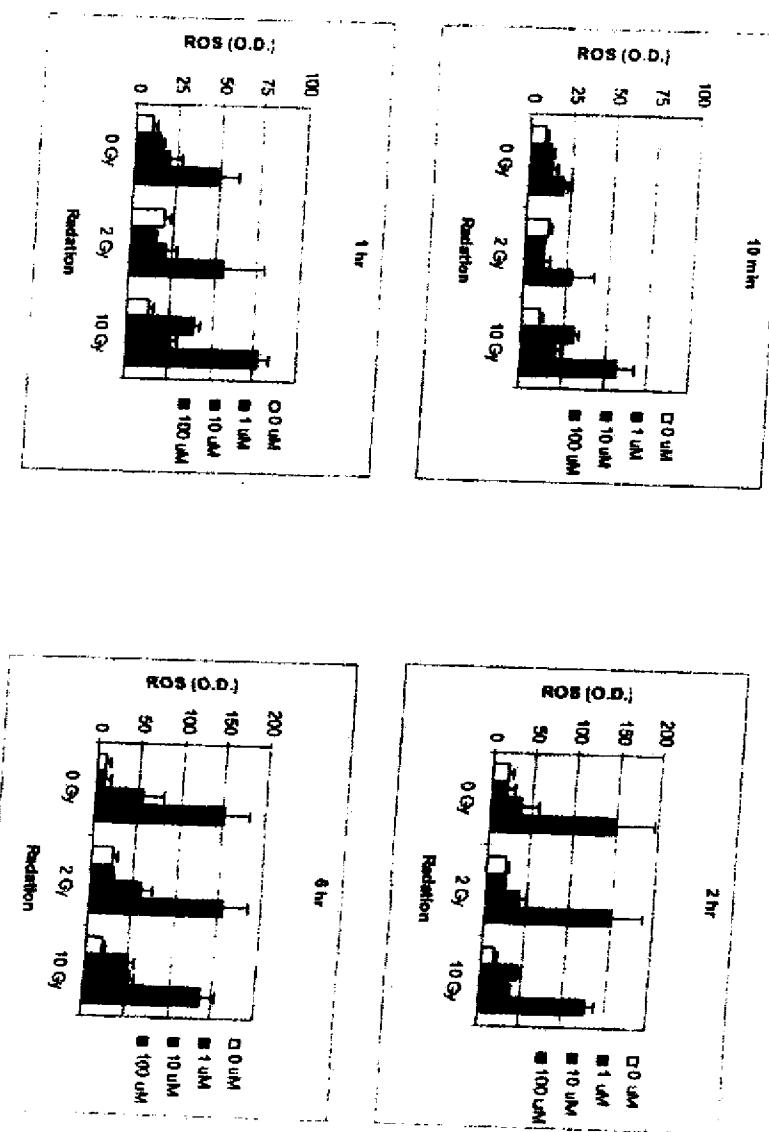
Figure 4
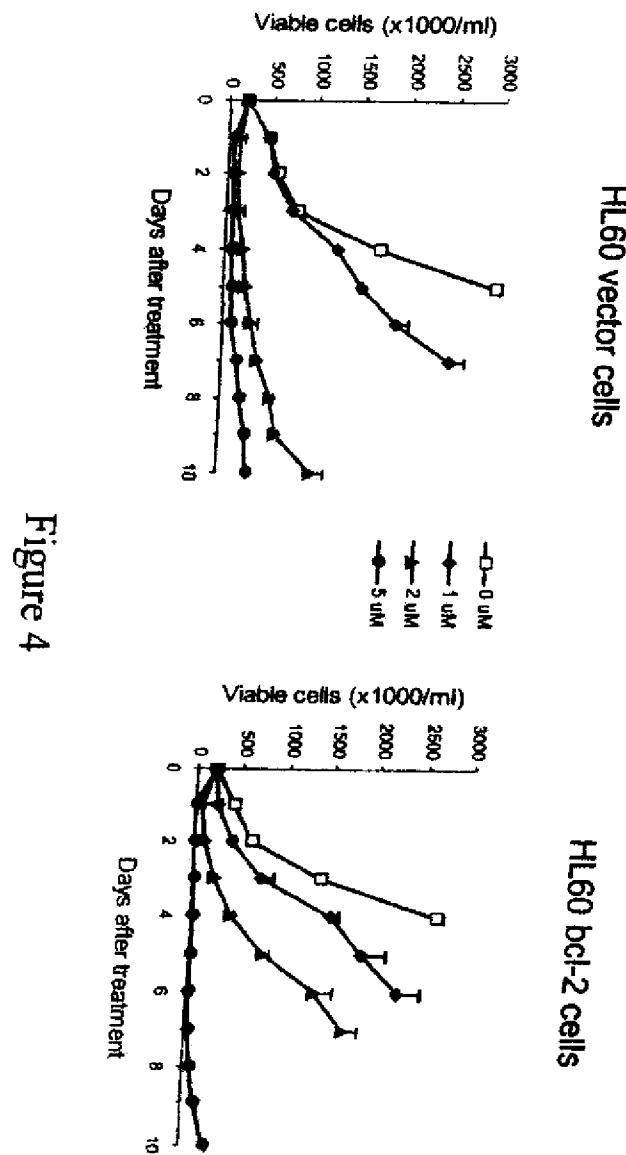
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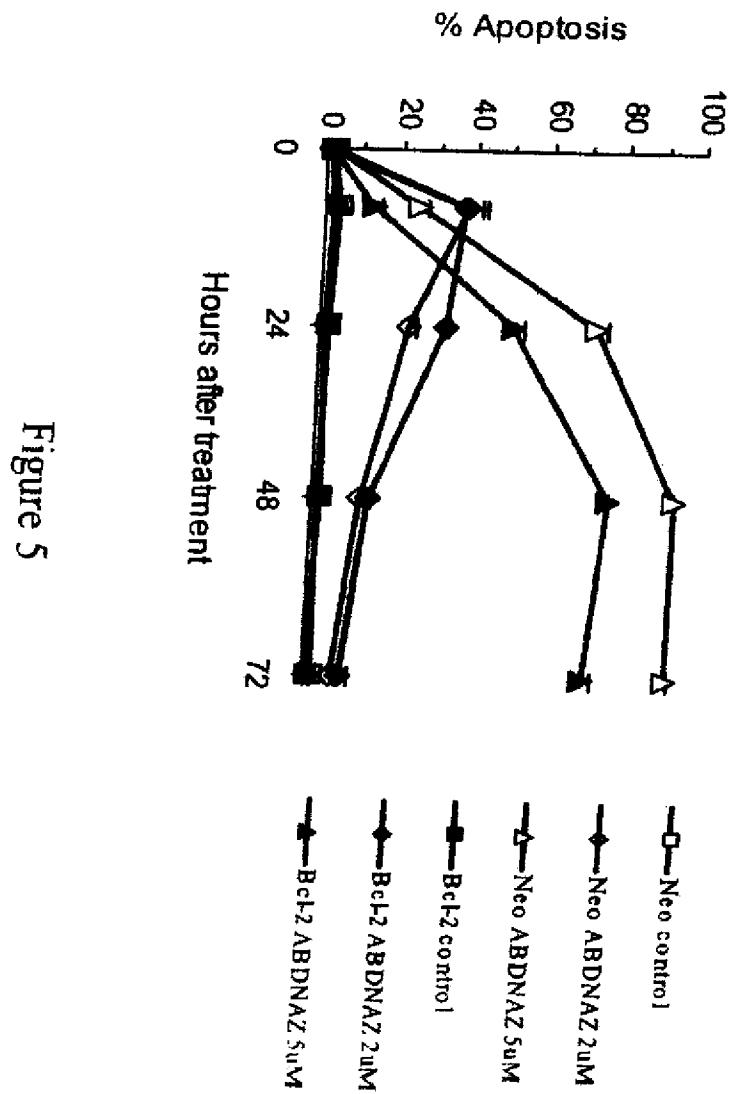
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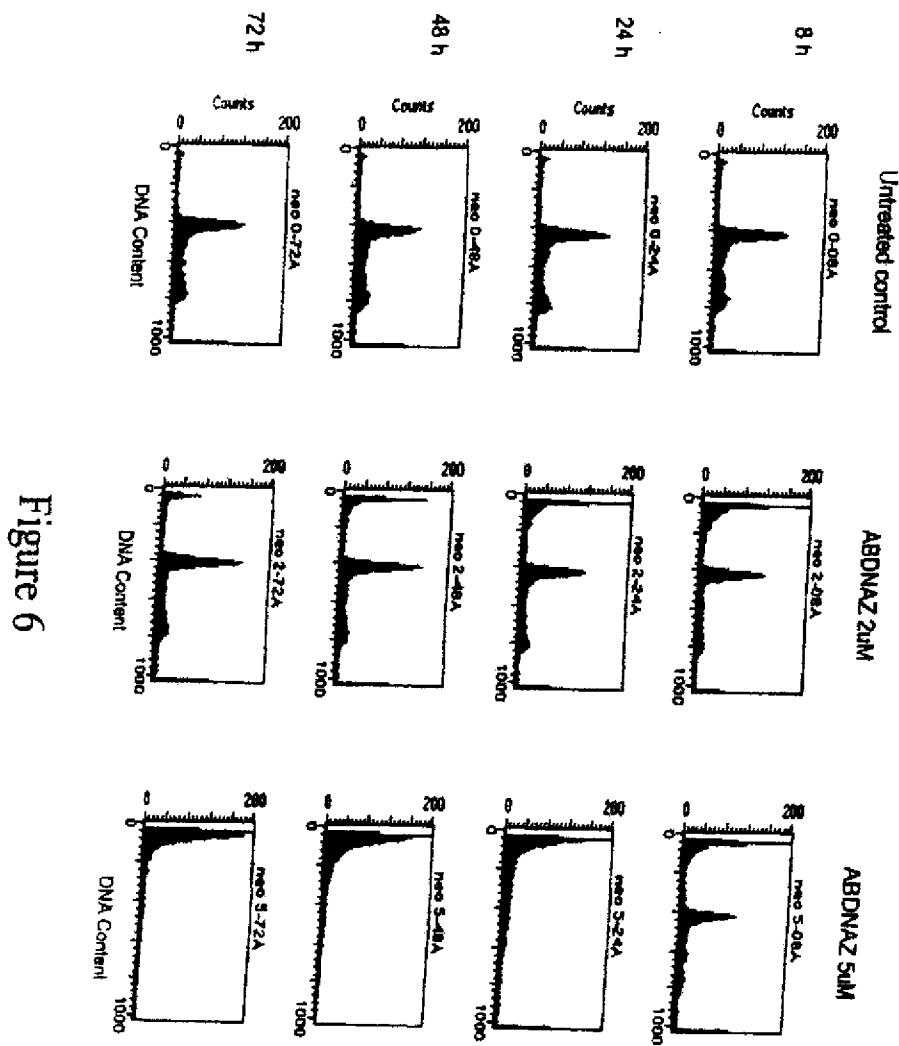
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Figure 6

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1-SF/7710698.1
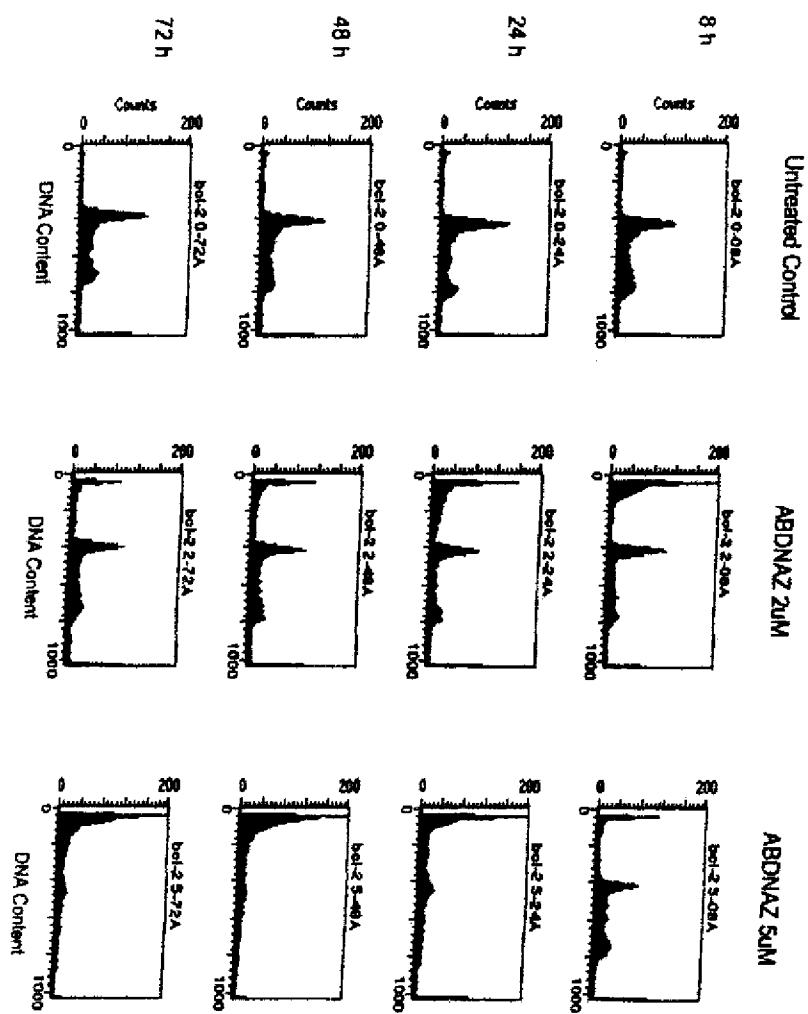
Figure 7

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Figure 8

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